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Rapid Method for Determining Mildew Susceptibility of Materials and Disinfecting Activity of Compounds

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A SIMPLE METHOD has been developed in the Quartermaster General Laboratories which minimizes the difficulties inherent in the tests used currently for evaluation of resistance of materials to microorganisms. In many cases tests can be completed overnight. Furthermore, the same apparatus can be used for testing a variety of materials, such as cotton, wool, leather, and plastics. Once the apparatus has been set up and adjusted for the particular test to be used, the method can be executed by the average technician. A brief description of the technique is given in this paper. Detailed experimental results involving various materials are being published elsewhere.

Millions of individual assays are conducted annually on the bactericidal and fungicidal activity of chemical compounds. Since the last war considerable emphasis has been laid on testing the susceptibility of various materials, such as cotton, wool, leather, paper, and plastics, to microorganisms for purposes of research and procurement. Here again the number of tests is very large.

One of the objectionable features of most of these testing procedures is the length of time required. In the case of cotton textiles the period varies between 14 and 45 days. For plastic films [5] about 30 days are required. Leather [2] demands an equally long period of incubation.

Another difficulty lies in the fact that different apparatus and conditioning rooms are required for different materials. In the case of cotton textiles, for example, the conditioning room is maintained at 70°F and 60% relative humidity; a Scott Tensile

Tester is used to determine the decline of fabric strength. In contrast to the conditions required for cotton textiles, plastic films are conditioned at 73°F and 50% relative humidity; a Clark Flexibility Tester is employed to measure increased stiffness of the film.

The method proposed here is based upon a measurement of the growth of the microorganism by means of a manometric determination of the total oxygen absorbed during growth. This is the basic difference between this method and those manometric methods previously proposed [1, 4, 6], which involve a measurement of the effect of a particular substance upon the respiration of a given quantity of organisms. A basic shortcoming of such techniques is that growth can be inhibited by concentrations of substances which may have little or no effect upon the immediate respiration. The converse situation has not been observed. Since the main interest is in preventing growth, the difficulty is obvious. It should also be pointed out that it would be easy to overlook an effective compound by relying upon such methods. In the method described here the cumulative respiration of the mass of organism which has grown in the presence of the inhibitor is measured. The basic assumptions are: (1) the amount of growth in a given period is a function of the effect of the inhibitor; (2) the cumulative respiration of the organism during growth is determined to a much greater degree by the quantity of organism than by the effect of the substance on respiration.

TABLE I. MILDEW-RESISTANCE OF TREATED COTTON FABRICS AS DETERMINED BY 2 TESTING METHODS

Pure-culture method			Manometric method		
Time (days)	% copper naphthenate as copper	Strength loss (%)	Time (hrs.)	% copper naphthenate as copper	Pressure change (mm.)
3	0.00	45	20	0.00	53
	0.01	23		0.01	48
	0.04	7		0.04	22
	0.16	0		0.16	6
	0.64	0		0.64	2
7	0.00	87	30	0.00	114
	0.01	87		0.01	102
	0.04	32		0.04	50
	0.16	0		0.16	10
	0.64	0		0.64	1
11	0.00	100	40	0.00	192
	0.01	100		0.01	177
	0.04	90		0.04	73
	0.16	0		0.16	18
	0.64	0		0.64	4

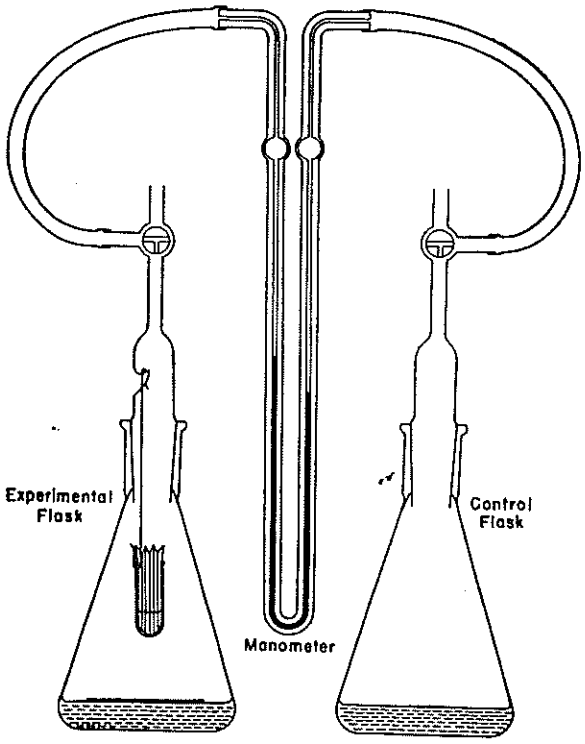

FIG. 1. *Differential manometer for evaluation of microbiological susceptibility.*

The apparatus (Figure 1) is, essentially, a Barcroft differential manometer. The manometer proper is constructed of capillary tubing of 2 mm. bore, with arms about 300 mm. long which are partially filled with Brodie's solution [3]. The bulbs have a capacity several times the volume of the manometric fluid. The culture vessels are 250-ml. Erlenmeyer flasks fitted with 24/40 standard tapered joints and attached to the manometer arms by flexible plastic tubing. Each flask contains 50 ml. of mineral-salts agar. Suspended in the flask is a cup containing 1.5 ml. of 10% potassium hydroxide and a filter-paper wick. In our laboratory the apparatus is placed in an incubator at 30°C. Actually, only the flasks need be in the incubator.

The method can be illustrated by the following procedure for cotton textiles. A fabric disc, 7 cm. in diameter, is placed on the agar surface in the experimental flask and inoculated with 1 ml. of a spore suspension of *Myrothecium verrucaria*, USDA 1334.2. Both experimental and control flasks are then attached to the manometer by the connectors (the

joints should be carefully sealed with stopcock lubricant). After an equilibration period of 3 hrs. the stopcocks are closed. During growth the organism absorbs oxygen from the gas space in the flask, which causes a decrease in pressure. The carbon dioxide produced is absorbed by the alkali in the well. Periodic readings are taken of pressure changes in the manometers. These changes are used as the criterion of susceptibility to mildew.

A comparison of data obtained by this method and by the standard pure-culture technique is provided by values given in Table I. Cotton khaki twill fabrics containing different amounts of copper naphthenate were used. The correlation between results obtained by the two procedures is excellent.

In tests of greater duration it is advisable to have wells containing alkali as well as the same agar medium in both experimental and control flasks. Both flasks are inoculated. Respiration due to growth of the organism on the agar, which contains significant quantities of assimilable impurities, is thus canceled out.

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